Kinetic analysis of the liver-type (GLUT2) and brain-type (GLUT3) glucose transporters in *Xenopus* oocytes: substrate specificities and effects of transport inhibitors

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We have expressed the human isoforms of the liver-type (GLUT2) and brain-type (GLUT3) facilitative glucose transporters in oocytes from *Xenopus laevis* via injection of *in vitro* transcribed mRNA. As reported previously [Gould, Thomas, Jess and Bell (1991) Biochemistry **30**, 5139–5145], GLUT2 mediates the transport of fructose and galactose, and GLUT3 mediates the transport of galactose. We have examined the effects of D-glucose, D-fructose and maltose on deoxyglucose transport in oocytes expressing GLUT2, and D-glucose, D-galactose and maltose on deoxyglucose transport in oocytes expressing GLUT3, and show that each sugar is a competitive inhibitor of transport. Moreover, D-glucose and maltose competitively inhibit fructose transport by GLUT2 and galactose transport by GLUT3, indicating that the transport of the alternative substrates for these transporters is likely to be mediated by the same outward-facing sugar-binding site used by glucose. Cytochalasin B is a non-competitive inhibitor of glucose transport by the well-characterized GLUT1 isoform. We show here that cytochalasin B is also a non-competitive inhibitor of the transport of deoxyglucose and alternative substrates by GLUT2 and GLUT3 expressed in oocytes. K_m and K_i values for each substrate and inhibitor are presented for each isoform, together with further analysis of the binding sites for alternative substrates for these transporter isoforms.

INTRODUCTION

The transport of glucose across the plasma membrane of animal cells is mediated by a family of facilitative glucose transporters, of which to date five different members have been identified, together with an expressed pseudogene-like sequence [1–3]. These transporters are the products of different genes, but exhibit high degrees of homology (between 45 and 65% identity at the amino acid level) and each isoform has a distinct tissue-specific pattern of expression. Secondary-structure predictions using hydropathy analysis have indicated that all transporters share a similar structure within the membrane, there being 12 putative transmembrane helices, hydrophilic domains between transmembrane helices 1 and 2, and helices 6 and 7, and intracellularly disposed N- and C-termini [1–3].

Little is presently known about the regions of the protein responsible for substrate binding or the conformational change that reorientates the sugar-binding site from outward to inward facing. Moreover, although a wealth of kinetic information is available for glucose transport and the specificity of the erythrocyte-type transporter (GLUT1), relatively little is known about other transporter isoforms [4–6].

Recently, we and others have utilized the *Xenopus* oocyte expression system for the analysis of heterologously expressed glucose transporters [7–10]. The low endogenous glucose-transport rates in these cells, together with the ease of microinjection of mRNA make the oocyte an ideal system with which to undertake a kinetic analysis of the heterologously expressed transporter. This system offers advantages over transport analysis on the native cell type, by virtue of the fact that many cells express more than one isoform, hence complicating analysis. Moreover, the oocyte system offers an ideal system for analysis of structure–function relationships, utilizing mutagenesis.

Here we describe a kinetic analysis of two transporter isoforms, the liver-type glucose transporter (GLUT2) and the brain-type transporter (GLUT3). We have determined the K_m for transport of 2-deoxy-D-glucose (deGlc) and D-fructose for GLUT2 and deGlc and D-galactose for GLUT3. In addition, K_i values for maltose, D-glucose, D-fructose, D-galactose and the fungal metabolite cytochalasin B are presented, together with an analysis of the substrate-binding sites of these isoforms.

MATERIALS AND METHODS

Materials

Wild caught female *Xenopus laevis* were purchased from African Xenopus Facility (Noordhoek, Republic of South Africa). Collagenase, cytochalasin B, 2,5-anhydro-D-mannitol, maltose, D-fructose and D-galactose were from Sigma (Poole, Dorset, U.K.). SP6 polymerase, RNasin and nucleotides were purchased from Promega (Southampton, Hants., U.K.). D-[6-^{3}H]Galactose, D-[U-^{14}C]fructose and 2-deoxy-D-[2,6-^{3}H]glucose ([2,6-^{3}H]deGlc) were from Amersham International. Recrystallized ethylidine-glucose was the generous gift of Dr. Gustav E. Lienhard (Dartmouth Medical School, Hanover, NH, U.S.A.). All other reagents were as described previously [8].

Oocyte isolation and injection

Female *Xenopus laevis* were maintained at 18 °C on a 12 h light/dark cycle. Individual oocytes were dissected and stored in Barth's medium [88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.82 mM $MgSO_4$, 0.41 mM $CaCl_2$, 0.33 mM $Ca(NO_3)_2$, 5 mM Hepes/NaOH, pH 7.4, penicillin at 10 μg/ml and streptomycin at 10 i.u./ml] as described [8]. All subsequent procedures were performed in Barth's medium. Oocytes were injected with

Abbreviation used: deGlc, 2-deoxy-D-glucose.
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25–50 nl of RNA at various concentrations or with water as a control and incubated in Barth's medium at 18 °C for 24–96 h before assay; the medium was replaced every 12 h [8].

Plasmid construction and synthesis of mRNA

The human glucose-transporter constructs used for the preparation of synthetic mRNA have been described previously [3,8]. Linearized plasmid DNA was used as a template for mRNA synthesis. RNA synthesis was performed as described [7,8].

Hexose transport in oocytes

Transport of deGlc

Groups of five to ten oocytes were incubated in 0.5 ml of Barth's medium at pH 7.4 in 13.5 ml centrifuge tubes. Transport measurements were initiated by the addition of an aliquot of [2,6-^{3}H]deGlc to the concentration indicated in the Figures/Tables. The reaction was stopped after the requisite time interval by quickly aspirating the media and washing the oocytes with 3 ml of ice-cold PBS (150 mM NaCl, 10 mM sodium phosphate, pH 7.4) containing 0.1 mM phloretin, a potent transport inhibitor [11]. The oocytes were washed in this fashion twice more and dispensed to scintillation vials, one oocyte per vial. These three washes were completed within 30 s. Then 0.5 ml of 1% SDS was added to each scintillation vial, and the vials were incubated at room temperature for 1 h with agitation before addition of OptiPhase scintillant and measurement of radioactivity.

Transport of D-fructose and D-galactose

Transport of these sugars was determined exactly as for deGlc. Groups of oocytes (typically five to ten) were incubated in Barth's medium at pH 7.4 in 13.5 ml centrifuge tubes, and the transport-rate measurement initiated by the addition of the appropriate sugar/radiolabel to the media. Uptake was stopped by three washes in ice-cold PBS containing 0.1 mM phloretin, and the radiolabel in each oocyte determined as for deGlc transport.

Determination of intracellular non-phosphorylated sugar levels

Method I

The relative levels of non-phosphorylated deGlc, D-fructose and D-galactose were determined using the method of Lienzeller and McAvoy [12]. Briefly, groups of five oocytes were incubated in Barth's medium at pH 7.4 plus the radiolabelled sugars at the same concentrations and for the same times as those used in the kinetic analysis. After the indicated time, transport was stopped by three washes in ice-cold PBS containing 0.1 mM phloretin and 1.5 ml of distilled water at 90 °C added to the tube. Oocytes were disrupted by trituration in a Pasteur pipette and incubated at 90 °C for 3 min. Then 1.5 ml of 0.1 M $ZnSO_4$ was added and the tubes were vortexed. Subsequently, 1.0 ml of 0.15 M $Ba(OH)_2$ was added, and mixed. Tubes were centrifuged at 500 rev./min in a bench-top centrifuge and samples of the supernatant counted for radioactivity. The supernatant contains only non-phosphorylated sugar species [12]. The relative contribution of non-phosphorylated sugar to the total sugar (i.e. deGlc and deGlc 6-phosphate) was calculated by measuring total sugar associated per oocyte exactly as for transport (above). Recoveries were typically between 90 and 95%.

Method II

The procedure used to determine the absolute concentration of non-phosphorylated and phosphorylated sugars was that of Olefsky [13]. Oocytes were incubated with sugars containing the radiolabel exactly as for transport assays. After the required time, oocytes were washed in ice-cold PBS to stop transport (as above) and 1 ml of water was added. The oocytes were placed in a boiling water bath for 20 min and the solution was then passed over 1 ml Dowex 1 × 8 in mini-columns. The columns were washed with 4 ml of water and the eluate was collected (contains the non-phosphorylated sugars). The columns were then washed with 8 ml of buffer containing 0.5 M ammonium acetate and 0.2 M formic acid, pH 4.9, and the eluate containing the phosphorylated sugars was collected. Portions from each sample of eluate were counted for radioactivity. In all experiments, recoveries (i.e. fraction of the radiolabel recovered from the columns compared with radiolabel associated with the oocytes measured exactly as for transport) were greater than 85%.

RESULTS

The K_m values for deGlc transport by GLUT2 and GLUT3 were determined by measuring the rate of transport over a range of sugar concentrations. Lineweaver–Burk analysis of a typical experiment is presented in Figure 1. Note that oocytes were exposed to deGlc for either 15 min or 30 min. Over these times we have shown that the amount of label accumulated within the oocyte increases linearly with time, suggesting that transport and not phosphorylation of the sugar is rate-limiting. This proposal was confirmed by measuring both the relative level and absolute levels of phosphorylated and non-phosphorylated sugars accumulated within the oocyte under the conditions used for analysis. In no case was a significant portion of the radiolabel associated

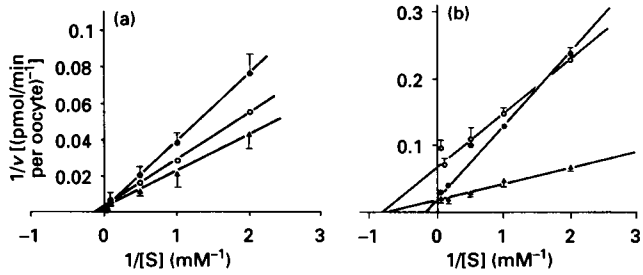

Figure 1 Lineweaver–Burk plot of deGlc transport rate in the presence of cytochalasin B or maltose for (a) GLUT2 and (b) GLUT3

(**a**) Each point is the mean of the transport rate determined from five oocytes as described in the Materials and methods section. Uptake was determined by 15 min exposure of the deGlc to the oocytes and the counts per oocyte determined as described. In all experiments, parallel incubations were carried out in oocytes that were not injected with mRNA ('control oocytes'), and the values obtained from this oocyte population subtracted from the values obtained from injected oocytes to obtain the true heterologous transport rate. Transport rates in 'control oocytes' were typically 5–10% of injected oocytes. In the representative experiment shown, ▲ represents the plot obtained in the absence of inhibitors, ○ in the presence of 4 μM cytochalasin B and ● in the presence of 150 mM maltose. For clarity, not all data points have been presented. Error bars are included for only two of the conditions for clarity. (**b**) A representative experiment is shown, performed exactly as described for (**a**) except GLUT3-injected oocytes were used: In the representative experiment shown, ▲ represents the plot obtained in the absence of inhibitors, ○ in the presence of 4 μM cytochalasin B and ● in the presence of 150 mM maltose. Error bars are included for only two of the conditions for clarity.

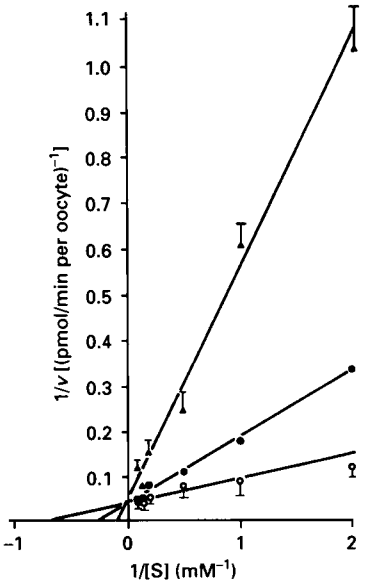

Figure 2 Lineweaver–Burk plot of deGlc transport mediated by GLUT3 in the presence of D-glucose or D-galactose

Each point is the mean of the transport rate determined from five oocytes as described in the Materials and methods section. Uptake was determined by 15 min exposure of the deGlc to the oocytes and the counts per oocyte determined as described. In all experiments, parallel incubations were carried out in oocytes that were not injected with mRNA ('control oocytes'), and the values obtained from this oocyte population subtracted from the values obtained from injected oocytes to obtain the true heterologous transport rate. Transport rates in 'control oocytes' were typically 5–10% of injected oocytes. In the representative experiment shown, ○ represents the plot obtained in the absence of inhibitors, ▲ in the presence of 10 mM D-glucose and ● in the presence of 20 mM D-galactose. Error bars are included for only two of the conditions for clarity.

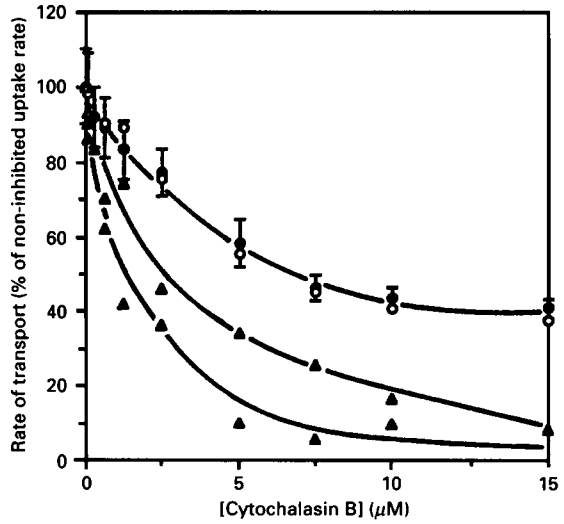

Figure 3 Dose–response curve of cytochalasin B on the inhibition of transport rates in GLUT2- and GLUT3-injected oocytes

Oocytes expressing GLUT2 were assayed for deGlc and D-fructose transport, and oocytes expressing GLUT3 assayed for deGlc and D-galactose transport as described. Transport rates were determined in the absence or presence of cytochalasin B in the bathing media at the indicated concentrations. Groups of five oocytes were assayed as described in the Materials and methods section, and the mean rate per oocyte is presented. Transport rates are expressed as a percentage of the rate obtained in the absence of cytochalasin B. ●, deGlc by GLUT2; ○, D-fructose by GLUT2; ▲, deGlc by GLUT3; △, D-galactose by GLUT3. For clarity, error bars have been included only for one of the curves, the errors in the other experiments were similar.

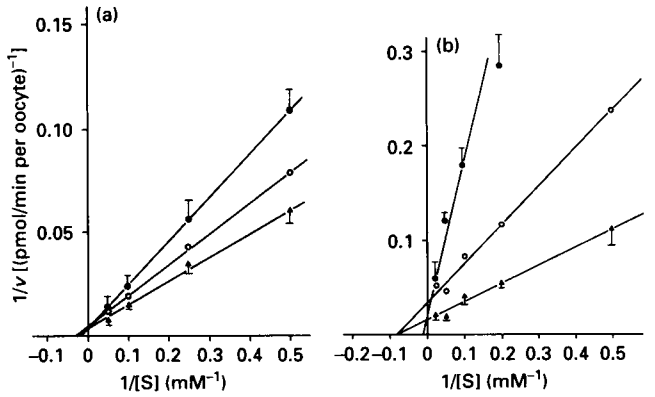

Figure 4 Lineweaver–Burk plot of rate of transport of D-fructose or D-galactose in the presence of cytochalasin B or maltose for (a) GLUT2 and (b) GLUT3

(**a**) Transport rates of D-fructose into oocytes expressing GLUT2 were determined as described. Each point is the mean of five separate oocytes at each concentration of sugar. Values presented are corrected for the transport of fructose by non-injected oocytes as described above for deGlc. In the representative experiment shown, ▲ represents the plot obtained in the absence of inhibitors, ○ in the presence of 4 μM cytochalasin B and ● in the presence of 150 mM maltose. For clarity, not all data points are presented and error bars are included for only two of the conditions. (**b**) Transport rates of D-galactose into oocytes expressing GLUT3 were determined as described. Each point is the mean of five separate oocytes at each concentration of sugar. Values presented are corrected for the transport of fructose by non-injected oocytes as described above for deGlc. In the representative experiment shown, ▲ represents the plot obtained in the absence of inhibitors, ○ in the presence of 4 μM cytochalasin B, and ● in the presence of 50 mM maltose. Error bars are included for only two of the conditions for clarity.

with the oocyte due to non-phosphorylated sugar. Even at the highest concentration of sugars used in the kinetic analysis, the level of non-phosphorylated deGlc accounted for less than 9% of the radiolabel associated with the oocytes for GLUT2 and less than 7% for GLUT3 (results not shown). Calculation of the intracellular concentration of non-phosphorylated deGlc indicated that, at all concentrations of deGlc used in the kinetic analysis, the concentration of deGlc inside the oocyte was less than 10% of that in the bathing medium. Similar results were obtained for D-fructose and D-galactose (results not shown). Taken together, we therefore believe that the rate of accumulation of these sugars in the oocyte is a true reflection of their transport rate, and the phosphorylation of the sugar by hexokinase is not rate-limiting under the conditions used herein.

The data in Figure 1 show a typical Lineweaver–Burk analysis of deGlc transport by GLUT2 and GLUT3 in the presence and absence of the transport inhibitors cytochalasin B (proposed to act at the inward-facing glucose-binding site) and maltose (proposed to interact with the transporter in the outward-facing conformation). The results indicate that cytochalasin B is a non-competitive inhibitor of deGlc transport by GLUT2 and GLUT3. Maltose (Figure 1) and D-glucose (Figure 2) exhibit the characteristics of competitive inhibition for both isoforms, as does D-galactose on GLUT3 (Figure 2) and D-fructose on GLUT2 (results not shown). The effects of increasing concentrations of cytochalasin B on the transport of deGlc and D-fructose by GLUT2 and deGlc and D-galactose by GLUT3 are presented in Figure 3.

We have also examined the kinetic profile of these inhibitors on the transport of alternative substrates for each isoform, specifically D-fructose transport by GLUT2 and D-galactose transport by GLUT3, and results of a typical experiment are

Table 1 Kinetic parameters for transport of sugars by GLUT2 and GLUT3

K_m and K_i values were determined from Lineweaver–Burk plots such as that shown in Figure 1. All values are means ± S.D. from between three and ten separate experiments for each condition. n.d., Not determined.

			K_i (mM)			
Transporter	Substrate	K_m (mM)	Maltose	D-Glucose	D-Fructose	Cytochalasin B
GLUT2	DeGlc	11.2 ± 1.1	125 ± 24	6.5 ± 1.3	204 ± 35	6.9 ± 1.2
	D-Fructose	66.7 ± 18.3	116 ± 5.8	15.3 ± 4.3	n.d.	6.1 ± 1.1
	D-Galactose	85.5 ± 10.7	n.d.	n.d.	n.d.	n.d.
			K_i (mM)			
Transporter	Substrate	K_m (mM)	Maltose	D-Glucose	D-Galactose	Cytochalasin B
GLUT3	DeGlc	1.4 ± 0.06	37.0 ± 1.8	1.6 ± 0.4	15.5 ± 4.5	2.1 ± 0.47
	D-Galactose	8.5 ± 2.5	29.2 ± 9.0	6.9 ± 0.3	n.d.	2.8 ± 0.72

Table 2 Effect of 2,5-anhydro-D-mannitol and 4,6-ethylideneglucose on transport of deGlc and D-fructose by GLUT2

Groups of five oocytes were incubated in 450 μl of Barth's medium containing either no additions (control), 10 mM or 50 mM 2,5-anhydro-D-mannitol or 50 mM 4,6-ethylideneglucose for 5 min. DeGlc (0.1 mM, 0.5 μCi per tube) or D-fructose (0.1 mM, 1.0 μCi per tube) was added and transport rates were determined as described. The experiment shown is representative of three separate experiments and the values are means ± S.D. of the rate of transport into five oocytes.

	Transport rate (pmol/min per oocyte)	
	DeGlc	D-Fructose
Control	3.2 ± 0.25	0.91 ± 0.14
10 mM Anhydromannitol	2.5 ± 0.20	0.80 ± 0.13
50 mM Anhydromannitol	1.4 ± 0.21	0.39 ± 0.10
50 mM Ethylideneglucose	1.8 ± 0.19	0.61 ± 0.10

Table 3 Effects of D-fructose and D-galactose on deGlc transport by GLUT2 and GLUT3

Groups of five oocytes injected with GLUT2 mRNA or GLUT3 mRNA were incubated in 450 μl of Barth's medium containing the sugars indicated at a concentration of 50 mM. DeGlc (0.1 mM, 0.5 μCi per tube) was added and transport rates were determined as described. The experiment shown is representative of three separate experiments and the values are means ± S.D. of the rate of transport into five oocytes.

	Transport rate (pmol/min per oocyte)	
Competing sugar	GLUT2	GLUT3
L-Glucose	2.61 ± 0.35	2.11 ± 0.25
D-Galactose	2.25 ± 0.15	0.50 ± 0.10
D-Fructose	1.60 ± 0.10	1.72 ± 0.15
D-Glucose	0.30 ± 0.05	0.11 ± 0.02

presented in Figure 4. The results of a series of such experiments are presented in Table 1.

The effects of other sugars on the transport of deGlc and alternative substrates by GLUT2 and GLUT3 have also been examined. The data in Tables 2 and 3 show the effects of a series of sugar analogues on the rates of transport of deGlc and D-fructose by GLUT2 and deGlc and galactose by GLUT3.

DISCUSSION

The advent of molecular biology, and its application to the study of membrane transport proteins, has resulted in a rapid advance in our understanding of how molecules are transported across the plasma membrane of animal cells. The isolation and heterologous expression of cDNAs encoding five members of the human glucose-transporter family has greatly enhanced our understanding of the regulation of whole-body glucose homoeostasis [1–3,7–10,14]. However, information on the regions of the transporter proteins that mediate the movement of glucose across the membrane remains to be gathered. Kinetic analysis has suggested that the transporter exists in one of two conformations, either with the substrate-binding site facing outward from the cell or facing inward with the substrate-binding site reorientated so as to face the cell cytoplasm (the so-called 'alternating conformer' model) [4,15–18]. Thus the transport of glucose across the cell membrane requires substrate binding at the outward-facing site, a conformational change to reorientate the substrate to the inward-facing site, and subsequent dissociation of the sugar from the binding site. Most, but not all (see for example ref. [19]), kinetic data available for GLUT1 are consistent with this model, and it is assumed that the other transporters utilize a similar kinetic mechanism.

The potential for structure–function analysis via the introduction of point mutations or large 'domain-swap' constructions should prove invaluable in determining the regions of the transporter associated with catalytic activity, such as the substrate-binding sites. One of the long-term aims of this laboratory is to utilize these approaches, coupled to heterologous expression in *Xenopus* oocytes. As a first step towards this goal, it is important to first establish the kinetic and functional properties of the native transporters. Here, we have undertaken an analysis of the liver-type transporter (GLUT2) [20,21] and the brain-type transporter (GLUT3) [22]. These transporters are kinetically the least well characterized of the transporter family, since many studies of the erythrocyte-type transporter (GLUT1) and the adipose/muscle-type transporter (GLUT4) have already been undertaken in their native cell types.

Previous work from this laboratory has demonstrated that, as

well as transporting glucose, GLUT2 is capable of mediating the transport of both D-galactose and D-fructose, and that GLUT3 mediates the transport of D-galactose [8]. Here we have extended these observations, and have determined the K_m values of transport of these sugars (Table 1). GLUT2 has a K_m for deGlc of about 11 mM, and GLUT3 a K_m of 1.4 mM. These values compare with those obtained for 3-*O*-methyl-D-glucose of 45 and 10 mM respectively (determined under equilibrium exchange conditions) [8]. Thus it is interesting to note that the relative affinities of the two analogues for these isoforms are similar, i.e. GLUT3 has about a 4–6-fold higher affinity than GLUT2.

We have further measured the K_m values for transport of D-fructose and D-galactose by GLUT2 and D-galactose by GLUT3. The data show that, for GLUT2, the relative affinities for the three transported substrates is deGlc > D-fructose > D-galactose (K_m values 11, 67 and 85 mM respectively). Similarly, for GLUT3, the K_m for D-galactose (8.5 mM) is much higher than that for deGlc (1.4 mM).

Cytochalasin B is a well-characterized inhibitor of glucose transport via the facilitative diffusion-type transporters [23]. It is well established that this inhibitor is non-competitive for glucose influx mediated by GLUT1, GLUT2 and GLUT4 [23–27]. Here we confirm the result obtained for GLUT2 and show that, as expected, deGlc transport by GLUT3 is also non-competitively inhibited by cytochalasin B. The K_i values are presented in Table 1, and show that, as previously reported, the K_i for cytochalasin B is higher for GLUT2 than for either GLUT1 or GLUT4 [27]. This is shown graphically in Figure 3. The value obtained for GLUT3 is intermediate between GLUT2 and GLUT1 [23–27].

We have further shown that the transport of D-fructose and D-galactose are non-competitively inhibited by cytochalasin B, and that the K_i values are similar to, if not identical with, those of deGlc (Figure 4 and Table 1). It is well established that GLUT1 and GLUT4 can be irreversibly photolabelled with cytochalasin B, and that this labelling may be prevented by D- but not L-glucose. In addition, photolabelling of GLUT2 has been reported, although under modified conditions [27]. We have also shown that GLUT3 can be photolabelled with cytochalasin B in the presence of L- but not D-glucose (C. A. Colville and G. W. Gould, unpublished work and [28]). There is a compelling body of evidence to suggest that this labelling occurs at, or close to, the inward-facing glucose-binding site (see e.g. ref. [4]). More convincingly, Deves and Krupka [29] have shown that glucose efflux is competitively inhibited by cytochalasin B, thus providing good evidence that cytochalasin B binds at or near the inward-facing sugar-binding site. Thus one interpretation of the results reported herein is that, since cytochalasin B exhibits similar K_i values regardless of the substrate, then the inward-facing substrate-binding site used by deGlc and D-galactose (GLUT3) or deGlc and D-fructose (GLUT2) are the same. However, this conclusion must be tempered with caution, since it does not necessarily follow that the site of cytochalasin B photolabelling and the site of cytochalasin B interaction as an inhibitor are the same [30].

We have examined the inhibition of the transport of these substrates by compounds likely to act at the outward-facing sugar-binding site, specifically maltose and D-glucose [18]. These compounds were determined to be competitive inhibitors of not only deGlc transport, but also of the transport of the alternative substrates for each isoform. The K_i values obtained are presented in Table 1. These observations are interpreted to imply that the alternative substrates tested in this analysis utilize the same outward-facing substrate-binding site as the preferred substrate (deGlc). This conclusion is further supported by the observations that D-fructose is a competitive inhibitor of deGlc transport by GLUT2 (results not shown) and D-galactose is a competitive inhibitor of deGlc transport by GLUT3 (Figure 2).

Given the somewhat surprising observation that GLUT2 could effectively transport D-fructose, we felt it important to try to further establish that fructose of deGlc transport by GLUT2 occurred via the same binding site on the transporter molecule. The most compelling evidence is that D-glucose is a competitive inhibitor of D-fructose transport and vice versa. However, in further support of this, we show (Table 2) that ethylideneglucose (a glucose analogue known to interact only with the outward-facing binding site) [18] and 2,5-anhydro-D-mannitol (a reduced fructose analogue) inhibited both deGlc and D-fructose transported by GLUT2. Although these data do indicate that the transport of D-fructose and D-glucose is mediated by the same binding sites on GLUT2, it is not clear which of the ring forms of D-fructose are transported. In aqueous solutions, D-fructose exists predominantly (about 70%) in the pyranose form; anhydromannitol is, however, locked in the furan form. Further studies are required to address this, but it is likely that the pyranose form of D-fructose is the preferred substrate.

It is also interesting to note that D-galactose is a more potent inhibitor of GLUT3 than GLUT2, and D-fructose is more effective at inhibiting transport mediated by GLUT2 than GLUT3 (Table 2), consistent with the relative K_m values reported above.

In summary, we report an analysis of the affinities of deGlc and a number of alternative substrates for the human liver-type (GLUT2) and brain-type (GLUT3) facilitative glucose transporters. These data show that GLUT3 has a significantly lower K_m for deGlc than that recorded for GLUT2. We have extended the analysis to include a determination of the relative affinities for D-fructose and D-galactose for GLUT2 and D-galactose for GLUT3, the major alternative substrates of these isoforms. It is demonstrated that cytochalasin B is a non-competitive inhibitor of the transport of deGlc and the tested alternative substrates for each isoform, and that the K_i values for inhibition of deGlc and the alternative substrates are similar. We have interpreted these results to suggest that the inward-facing substrate-binding sites used by deGlc and D-fructose (GLUT2) or D-galactose (GLUT3) are similar or overlap. We further show that D-glucose and maltose are competitive inhibitors of both deGlc and D-fructose transport by GLUT2, and deGlc and D-galactose transport by GLUT3, suggesting that all transported sugars are likely to use the same outward-facing sugar-binding site on the transporter molecule. This is further indicated by the observation that each of the alternative substrates for each isoform is a competitive inhibitor of deGlc transport and that D-glucose is a competitive inhibitor of the transport of the alternative substrates.

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